tinge at apex, sometimes the whole wings have a fuscous tinge; tegulæ black, with a dark red spot in the centre; nervures black.

♂. Similar, the whole of the abdomen punctured; the apical segment with eight teeth, two lateral, four apical above, and two below; the abdomen below, except the apical segment, is black, not red.

Hab. Deesa.

Length ♀ 8-11 mm., ♂ 7-9 mm.; Exp. ♀ 14-18 mm., ♂ 12-14 mm.

VIII.—*Studies in the Chemistry and Physiology of the Tea Leaf. Part I. The Enzymes of the Tea Leaf.*—*By* Harold H. Mann, B.Sc.

[Received November 27th; Read December 4th, 1901.]

The production of a food product from the leaves of plants is in actual practice of very rare occurrence. Except in the case of a few vegetables and potherbs, and of some leaves used only as narcotics and stimulants, it may be said not to exist except in the case of tea. And in the production of tea, if the type of leaf used, the method of collection, the induction by artificial means of a constant unnatural succession of young growing shoots be taken into consideration, the whole question becomes of so exceptional a character that a study of the chemical and physiological condition prevailing under such circumstances would probably be extremely interesting. If, in addition, such a study be combined with that of the changes which take place in the leaf after plucking until its conversion into black tea,—changes which result in profound alterations in the substances present and which altogether alter the commercial characteristics of these products, the matter becomes one of great economic importance. In the series of papers I hope to contribute to the Asiatic Society on this subject, and of which this is the first, I shall try, however, to very largely eliminate the direct economic interest, which will be reserved for another place and another occasion.

In order, however, to follow the subject it will be necessary to give a short account of the processes by which tea is produced. The tea leaf as used in this manufacture consists of the youngest leaf on the plant, and only the youngest two open leaves on each shoot together with the unopened leaf bud are now usually plucked. This necessitates, if a large amount of leaf is not to get too old for plucking, and hence to be wasted, that every bush should be gone over by an expert plucker about every seven days. Having obtained the leaf in this manner, it is allowed to wither—to lose its turgescence—by exposure in very thin layers to air as cool as possible until the whole has got to such a condition that on rubbing in the hand the leaves no

longer break, but are sufficiently pliable to roll up. At this stage it is rolled, a process whose effect is to break the cells of the leaf, allow the sap to spread itself over the surface, and so come in contact with air during the process of fermentation. This latter merely consists in exposing, for a time varying from two to six hours, the rolled leaf in thin layers in as cool and airy a room as possible. Marked changes here take place; the green leaf takes on a brown coppery colour and acquires an aroma totally different from that of fresh leaf. When sufficiently fermented,—which is judged at present entirely by appearance and smell,—the whole mass of tea is dried usually by a powerful current of hot air, sorted and put on the market.

It is evident that the changes important from our point of view principally takes place during withering and fermentation. Withering has usually been considered to be little else than a process of partial drying without the loss of pliability which would take place were the operation conducted at a high temperature, and the idea that profound chemical changes may take place has hardly been mooted. On the other hand, the speculations as to the nature and cause of the fermentation process have been legion. In the early days it was usually considered to be merely incipient putrefaction, and this idea was supported by the fact that a slightly longer exposure than that given leads to an intensification of the brown colour, to the development of increased acidity and ultimately to putrefactive decomposition.

Prior to the experiments of Mr. Bamber,* the statements made rested on no experimental basis. His work however has revolutionised the ideas on the subject. He maintained (1) that very few organisms were present, and the time was too limited for their development in quantity, and that hence the process could not be caused by bacteria, (2) that the fermentation will not take place in absence of oxygen, even if the oxygen was replaced by carbon dioxide, (3) that a large quantity of air is required, (4) that after heating the leaf with dry steam for a few minutes the fermentation proceeded normally. Hence he maintained that the so-calld fermentation process was not a fermentation at all, but was due merely to the direct chemical action of the atmospheric oxygen on the constituents of the juice exposed in thin layers, and he hence substituted the term "oxidation" for "fermentation" in naming the process.

In this position the question remained, except for mere speculative opinions,† until the beginning of 1900, when Mr. Bamber returned to the question, and to a certain extent revised his former opinion. He

* Chemistry and Agriculture of Tea, 1893.

† See, for instance, D. Crole, Journal of the Society of Arts.

then wrote as follows:* "Quite recently I have succeeded after numerous attempts in isolating a minute proportion of a soluble oxidising ferment somewhat similar to the oxidases recently discovered in several plants of different natural orders. The substance in question, which evidently has a considerable bearing on the oxidising properties of the tea, apparently does not exist in the active form in the fresh green leaf, but is changed either during the withering if the leaf is bruised, or during the rolling processes when the various organic acids, etc., are liberated from the cells." This was, I believe, the first announcement of the discovery of a soluble ferment or oxidase in the tea leaf, and of course it meant that Mr. Bamber no longer attributed the changes which take place entirely to the oxidising action of the air independently of ferments of any kind.

Later in 1900 in a private communication to me, Mr. C. R. Newton of Kurseong stated that he had detected an oxidase in the leaf, but the observation was never published till a few weeks ago.† In the meantime Mr. Aso, a Japanese scientist, has published his discovery of the same ferment, but as I have not been able to get hold of the publication in which he announces his work,‡ I am unable to say to what extent he has carried his researches.

My own work was done by the courtesy of Messrs. Finlay, Muir & Co., the Agents, the Amalgamated Tea Estate Company, Ld., the Owners, and Mr. J. D. Gwilt, the Manager of the Moondakotee Tea Estate, Darjeeling, on that estate, during the past tea-making season.

In trying to ascertain the nature of the changes which occur during the manufacture of tea leaf, it seemed of primary importance to determine to what extent, if any, bacterial action intervened, especially as Mr. Bamber's experiments were not quite convincing on the subject. For this purpose it was necessary to cultivate any organisms which might be present on a medium which would as far as possible eliminate the ordinary putrefactive bacteria and only allow those which could have any effect on the tea leaf to grow. This at once puts out of court such common media as peptogelatin, peptone-agar-agar, or any similar preparations as the basis of cultivation in which, as a matter of fact, a large number of putrefactive organisms (many of them of the *Bacillus subtilis* type) do actually grow when fermenting tea is placed in contact with them. The medium finally adopted consisted of tea leaf itself ground up finely, and then placed in small patches 1 to 1½ inches in

* Report on Ceylon Tea Soils. Colombo, 1900.

† Indian Gardening and Planting. November 7th, 1901.

‡ Bulletin of the Imperial College of Agriculture, Tokio. 1901. Vol. 4, page 254.

diameter inside a petri dish, and sterilised. A slight change of colour took place during sterilisation, but afterwards none, and dishes so prepared could be kept for weeks. The sterilised tea leaf thus obtained was then inoculated with fermenting leaf, and in about two days colonies were evidently appearing. After three days' culture, these were examined and inoculated with sterilised tea juice, and after a further three days' growth there, the cultures of the second generation were utilised. In every case only one organism was certainly found. It produced colonies consisting of yellowish brown slimy masses without shape, and raised up like drops from the mass of the sterilised tea leaf. In texture these colonies were sticky and a little ropy. Under the microscope the organism was found to be a small bacillus about $1 \cdot 2\ \mu$ long and nearly $1\ \mu$ broad. A pure culture having been obtained sterilised leaf was inoculated with a solution containing the organism in large amounts. No change whatever took place in colour in three hours,—the normal time of fermentation,—but a sour smell had developed. If freshly rolled leaf, instead of sterilised leaf, were used, the inoculated portion had taken on a sour smell in $1\frac{1}{2}$ hours, while the check experiment was equally coloured, but the fermentation was proceeding normally. The organism was evidently in fact one of the many lactic acid bacteria and had no part whatever in the normal process of fermentation. Inasmuch as this was the only microbe which could be isolated in this way, as it had no effect on the colouring of the tea leaf, and as it caused the leaf to become sour earlier than it would otherwise have done, one may, I think, take it as finally settled that microbial organisms play no essential part in the fermentation of tea, and that when present they are rather of the nature of impurities than essential factors in the process.

In the absence of bacteria capable of causing the changes observed during the fermentation of tea, it was natural to look for enzyme action, especially as during the past five years the effect of unorganised ferments has been discovered to be paramount in cases where their influence had hardly been previously suspected. The curing and fermentation of tobacco is an example. Here Oscar Loew* has shown that the changes taking place during both these processes are primarily due to the action of enzymes. But in attempting to isolate the active ferments in tea, one is met at the outset by a difficulty pointed out long ago by Brown and Morris† that it was very difficult to extract enzymes from vegetable tissues in presence of a solution containing tannin. Since the young tea leaf contains twenty per cent. of tannin

* Reports of the U.S.A. Department Agriculture. Nos. 59, 60 and 65, 1899-1901.

† Journal of the Chemical Society. 1893.

(calculated on the dry matter) the difficulty was especially great in the present case. The method finally adopted for isolating and at a latter date for estimating the amount of oxidising enzyme present was as follows:—10 grams of fresh (or 6-6 grams of withered leaf) were ground up in a mortar till they formed a pulp, and in each case 5 grams of hide powder (pure for analysis) were added and the mass again ground thoroughly together. 50 cubic centimeters of water were now thoroughly incorporated with the mixture and the whole left for two hours. At the end of this time the mass was filtered quickly through cloth, with pressure, and the residue washed twice with water. It was found that practically the whole of the extractable part of the oxidising enzyme was thus removed. The liquid obtained was now mixed with four times its volume of alcohol, which precipitated the whole of the enzyme. After settling thoroughly, the precipitate was filtered again through cloth, and to the residue 25 to 30 c. c. of water were added. The whole of the enzyme was thus obtained in a small volume which on filtration gave a clear liquid in which various tests could be made.

The standard test for oxidising enzymes or oxidases is that with guaiacum resin. If an alcoholic solution of this resin be mixed with a liquid containing one of the class of substances under discussion, a blue colour varying in intensity with the quantity of enzymes present will appear after two or three minutes. With the solution from tea leaf prepared as above, this reaction was obtained immediately, and if further a drop or two of Hydrogen Peroxide were added the reaction became very much more intense. It was hence at first supposed that two enzymes were present, the one giving a blue colour without Hydrogen Peroxide, the other only producing the reaction in its presence. If this were the case they ought surely to have different resistances to heat, and by this means one ought to be able to separate them. This was found, however, not to be the case. A solution in water containing the oxidases was exposed to various temperatures in each case for three minutes, with the following results:—

Temperature.	Reaction with Guaiacum Resin.	
	Without Hydrogen Peroxide.	With Hydrogen Peroxide.
60°C.	Just as intense as before heating.	Just as intense as before heating.
70°C.	Do.	Do.
80-81°C.	Slight decrease in intensity of reaction.	Reaction distinctly lower in intensity.
83-85°C.	Reaction practically disappeared.	Reaction almost disappeared.

There is therefore no difference in the sensitiveness of the substances producing the two reactions to heat, and both are destroyed by an exposure for three minutes in aqueous solution to 83 to 85° C. and the destruction commences below 80° C. One may therefore say,—and the conclusion is confirmed by the result of a large number of other attempts (to be afterwards referred to) to isolate the two apparently different enzymes,—that we have no evidence of the presence of more than one ferment oxidising guaiacum in the tea juice. I am inclined to attribute the difference in reaction to the presence of part of the ferment in the juice as zymogen or pro-enzyme, which is brought into definite action by the Hydrogen Peroxide. The distinction between the reactions with and without Hydrogen Peroxide, therefore, remains a convenient one, and I have kept it up throughout the present work.

To estimate the amount of these enzymes 5 c c of the clear liquid prepared as above, were mixed with an equal volume of alcohol, and then 10 drops of a solution of guaiacum resin in alcohol added, and the colour measured in a Lovibond's tintometer. The measurement must always be made at the same length of time after the addition of guaiacum tincture. After the intensity of the colour has been noted, ·5 c.c. of a 10 volume solution of Hydrogen Peroxide were added and the colour again measured. The intensity of colour gives a rough measure of the relative amount of enzyme in the several cases, and has been utilised for this purpose throughout the present work. It depends on the fact that the colour given is all but absolutely a pure blue, and hence one can neglect the amount of any other colour which may be present in the liquid, and merely take the intensity of the blue as showing the relative amount of the oxidase.

It seems almost impossible to prepare the enzyme pure in a dried condition. If the clear solution prepared as above be reprecipitated with alcohol, a mass is produced very active towards guaiacum solution, etc., but if an attempt is made to dry the precipitate at a low temperature its oxidising power rapidly diminishes, and when dry there is hardly any reaction left. The whole of the reactions had therefore to be studied in the solution prepared as above, which in addition to the enzyme contained a certain proportion of gummy, pectic and saline matters.

The oxidase was very sensitive to the action of acids. A solution of the enzyme was immediately rendered absolutely ineffective in a solution containing ·4 per cent. of Sulphuric Acid. ·04 per cent. had, however, only very slight effect after 2 hours. 3 per cent of Acetic Acid destroyed the ferment entirely in 2 hours. By Alkalies it was less affected but still was rapidly destroyed. 3 per cent. Ammonia nearly destroyed all action after $4\frac{1}{2}$ hours. Caustic Potash of the same strength had little

effect after 2 hours, but after 4 hours only a slight reaction was obtained until Hydrogen Peroxide was added, when a fairly intense blue colour was produced with guaiacum tincture. After 18 hours there was still the difference, though even with Peroxide the colour was much less intense. This is a strong indication that the latter reagent liberates the enzyme from a compound (a pro-enzyme) in which it was much less easily attacked by Alkalies than when already free.

That we have here to deal with an oxidising enzyme was made clear by its action with hydroquinone and with pyrogallol. In the former case darkening, indicating oxidation, was very rapid in presence of the enzyme, and much more so than in check solutions to which either no addition was made, or to which even a boiled solution of the ferment had been added. I was not able to isolate the product of oxidation. The same rapid darkening took place in presence of the enzyme with pyrogallol. In three hours the colour had become very dark brown, while both the duplicates were hardly tinted brown. After 18 hours the difference was extreme, the pyrogallol being almost entirely oxidised in the one case, only a light brown colour having been produced with boiled ferment or with none at all. Gallotannic Acid behaved differ-ently and showed itself far more resistant to oxidation than either of the above substances, very slight change having taken place even after 18 hours.

The reaction with Hydroquinone was so striking that it was used to determine the optimum temperature for the activity of the enzyme. Three solutions of Hydroquinone were prepared. No. 1 was kept at ordinary temperature (26° C.) No. 2 at 50-55° C. No. 3 at 60-62° C. After $1\frac{1}{2}$ hours No. 1 was hardly changed, while oxidation was proceeding rapidly in Nos. 2 and 3 and no difference could be detected between them. After 4 hours however while No. 1 still showed hardly any alteration, No. 2 was far and away ahead of No. 3 in the progress of the reaction. It was regrettable that there seemed no means of measuring exactly this progress, but the experiment clearly shows that the best temperature for the action of the ferment does not exceed 53° C. and that it is much more rapid at this temperature than at the usual temperature at which the operation is carried out.

The crucial test, however, as to the relation of this oxidase to the fermentation of tea was whether when a solution of the enzyme was added to tea juice, the colour which forms the mark of fermented tea was produced more quickly than in a normal case. An experiment on this ine was therefore made, and the colour was produced considerably more quickly than in the untreated juice. An attempt was made to utilise sterilised tea juice for this purpose, but the process of sterilising to destroy the enzyme (as the ferment cannot be removed by any filtra-

tion method) induced such profound changes in the tea juice that it was impossible to make the absolute test which this method would have given. When a more rapid colouring of tea juice or tea leaf takes place in presence of an additional quantity of the enzyme, and at the same time proceeds normally, there seems no justification for doubting the essential connection of the oxidase isolated from tea leaf with fermentprocess.

The next point would naturally be to ascertain the part of the leaf richest in enzyme, and the leaf of the flushing shoot plucked for manufacture which contained the most. This was therefore determined by carefully separating the leaves from one another and from the stalk, and determining the oxidase in each separately by the method previously described. The following results were obtained, the amount present in the tip leaf being unity in each case, and the whole being calculated on the dry matter of the leaf.

	Relative amount of active enzyme.	Relative total amount of enzyme.
Tip unopened leaf ...	1·00	1·00
First open leaf ...	·65	·61
Second open leaf ...	·48	·80 (?)
Leaf stalk ...	1·64	1·39

This indicates a rapid decrease in the amount of enzyme present as the leaf becomes older, but that the stalk contains a good deal more than any other part of the shoot. The above figures much exaggerate this excess, however, owing to the fact that the stalk contains much more water than the leaf, and as a matter of fact in the fresh condition the tip leaf and the stalk contain about an equal amount of enzyme. It is interesting to compare the relation of various other constituents of the leaves to the enzyme as given above, and for the Acidity, the Tannin, and Phosphoric Acid, we have these as follows:—

	Total Acidity.	Acidity in the absence of Tannin.	Tannin.	Phosphoric Acid.
Tip leaf	1·00	1·00	1·00	1·00
First open leaf ...	·94	1·09	1·03	·88
Second open leaf ...	·94	1·06	·91	·75
Leaf stalk	·70	1·09	·86	·79

In each case the tip leaf is regarded as unity, and each is calculated on the dry matter. The acidity due to the tannin in each case amounted to about half the total acidity, using phenol phthalein as indicator, and appeared to be practically identical (on the dry matter) throughout the flushing shoot. The enzyme therefore appears not to bear any very close relationship to any of these constituents calculated as above, if the stalk be included, but if this be left out (as I think it may be, for it is to a great extent nothing but a channel of conveyance), then the enzyme will be found to follow both the Tannin and the Phosphoric Acid, but not the acidity, except that caused by the tannic acid.

The practical consideration now comes in as to the relation of this enzyme to quality in tea. The only means of ascertaining this was to compare the leaf from gardens lying near one another producing distinctly different types of tea, and teas which were regarded by experts as of different quality. It is necessary, of course, in face of the distribution of the enzyme in the flushing shoot above pointed out, that the leaf shall be of approximately the same type. For instance, a stalky tea eaf could not in any sort of fashion be compared with one giving little stalk. With this reservation, which was taken into account in the experiments which follow, the figures obtained seem to indicate that a large amount of ferment means a high quality tea, and a reduction in the enzyme present means a lowering of the flavour of the product. It is in the flavour that the effect is most marked, the strength of the tea being not nearly so much affected.

Three gardens are concerned in what follows. These are A, which, judged by market prices, has been making a medium Darjeeling tea: B, which has had the reputation of making about the best tea in the Darjeeling district for many years, and C, which has produced absolutely the highest value teas in the district during the past season.

A comparison was first made between a sample of leaf from A and two samples from bushes of different types from C, No 1 being an "Assam" type of plant, and No. 2 from a "China" type. The following figures were obtained :—

	Relative amount of active enzyme.	Relative total amount of enzyme.
A.	1·00	1·00
C. No. 1. ...	2·17	2·18
C. No. 2. ...	1.44	1·68

The amount of oxidase in A is here regarded as unity, and its type of plant was "China", and all figures are given on the dry matter of the leaf as plucked for manufacture. In each case a much larger amount of enzyme was present in the leaf which made the better tea.

From Garden B, I had three samples of leaf. No. 1 was from young "Assam" plant producing excellent tea, No. 2 was from a low level extension also of "Assam" plant but giving the worst tea in the garden though still quite as good as the district average, No. 3 was from "China" plant producing tea of very high quality. Comparing, in precisely the same manner as above, all these samples of leaf with that from garden A the following figures were obtained :—

	Relative amount of active enzyme.	Relative amount of active enxyme.
A.	1·00	1·00
B. No. 1	1·88	1·03
B. No. 2	1·17	1·32
B. No. 3 ...	1·83	1·32

In this case again it appears that the quality varies with the quantity of ferment present in the leaf in an active form. It will be noticed that the various amounts of enzyme are much closer together when the total, including the supposed pro-enzyme, is considered, than when the active form only is taken into consideration. It may well be that this difference is a real one, and that there is some cause in certain places from soil, climate or other consideration which may prevent the formation of active enzyme, and such a cause would affect the quality.

Another point remains in this connection. What effect has withering on the amount of ferment? The answer to this question has been exceedingly interesting, and seems to indicate that this operation possesses a function in the manufacture hitherto quite unsuspected, and which leads to a very different conception of the process to that hitherto held. The leaf from gardens A and B above considered, were allowed to wither, and taking full account of the corresponding loss of moisture, the enzyme again determined. Taking the oxidase in the fresh leaf at garden A as unity (that is to say that the unit in the last table is the same as unit in the following) we have :—

	Relative amount of active enzyme.	Percentage increase during withering.	Relative total amount of enzyme.	Percentage increase during withering.
A.	1·81	81·0	1·69	69·0
B No. 1 ...	2·49	31·9	1·87	43·8
B. Na. 2 ...	1·88	60·7	1·87	41·6
B. No. 3 ...	2·19	19·7	2·19	65·9

There remains to be considered the circumstances which cause the production of the oxidising enzyme by the plant. I have as yet only had the opportinity to touch upon one or two of these. It seemed probable however that the amount of light received by the plant would very materially influence the amount. Three bushes, side by side, were therefore taken, and one was so covered up for ten days, so that the leaf grew in darkness not quite suffcient to etiolate the young leaves. Leaf was plucked from all three bushes on the same day, in No. 1 as soon as it was light in the morning, in No. 2 (the darkened plant) soon afterwards, and in No. 3 late in the afternoon.

From the result it would appear that darkness favours the formation of the oxidase, and that there is a difference in this respect between the leaf gathered in the early morning and that obtained after a day's sunshine. In the leaf grown entirely in darkness the reserve stock or proenzyme seemed to have been increased, but that immediately active was rather lower than in the normally produced leaf. I intend to take up this line of investigation more thoroughly later on.

In a recent publication I have shown how dependent the quality of tea is on the amount of Phosphoric Acid in the soil. It is curious to find that this connection of flavour and Phosphoric Acid, according to the present experiments, seems to run parallel with the apparent connection between Phosphoric Acid in the soil (and also in the leaf,) and the amount of oxidising enzyme.

I give here the analyses of soil from the gardens A and C above mentioned, and it will be at once seen that the amount of Phosphoric Acid corresponds closely with the amount of enzyme in the leaf. I am disposed to insist on this point in view of the previously indicated relationship of quality, *i.e.*, flavour, to Phosphoric Acid in the soil.

	A.	C.
Phosphoric Acid	·061	·124

The only question remaining to be discussed with relation to the oxidase under consideration appears to be its localisation in the leaf and stalk. An attempt was made to determine its position by three methods. The first of these consisted in cutting the sections of leaf and stalk and ascertaining in what cells the brown colour commenced to form. In the leaf this always took place at definite points in the centre of the leaf. In the cells where the browning commenced there seemed to be on examination with a very high power in many cases a small irregular black body from which the browning radiated. This could only be seen where the sections are thin and consisted of little

more than one layer of cells. I have not been able yet to more exactly ascertain the nature of these small black bodies. The second means of ascertaining the whereabouts of the enzyme was to kill the leaf in chloroform vapour, when it became brown in a very few minutes, and then cut sections of the leaf and leaf stalk as before. In the leaf precisely the same occurred as was found by the first method,—the brown colouration always commenced at points in the centre of the tissue. In the stalk the result was very definite. Oxidation always occurred first just outside the fibro-vascular bundles, then it took place just inside the same layer, and thirdly the cells just inside the epidermis were attacked. A third method gave results quite agreeing with this as to the stalk, but no definite results were obtained with the leaf. In examining the sections by this method, they were first left 12 hours in alcohol to extract the tannin and precipitate the enzyme. They were then put in a drop of water on a slide, a drop of guaiacum tincture immediately added and the preparation then again washed with water. The blue compound is soluble in alcohol, and the enzyme is soluble in water so that it is necessary to do these operations as rapidly as possible.

The result obtained showed a general blueing of the section, but on leaving a short time in glycerine the parts to which the enzyme had merely spread faded, and left the rest quite heavily stained. The fibro-vascular bundles were quite free from blue colour, and as for the rest it was most intense first in the cells just outside this layer, second in the point just inside it, and third just inside the epidermis. So far as the stalk is concerned then, the several methods agree as to the points at which he greatest amount of enzyme is to be found, and this distribution is almost exactly the same as that of the largest quantity of the tannic acid.

In general, therefore, with regard to the question already considered it has been established—

(1) That an oxidase occurs in the leaf of the tea-plant used for manufacturing tea.

(2) That this oxidase is the principal agent in bringing about the fermentation and colouring of the leaf. It is most active below 55°C. and is destroyed about 80°C., is very sensitive to acids, and also to alkalies, but not to quite the same extent. There is distinct evidence that part of it usually occurs as a pro-enzyme in the leaf.

(3) That it occurs in greatest quantity in the unopened tip leaf of the shoot, and that the quantity decreases as the leaves get older, but that the stalk contains at least the same amount as the tip leaf.

(4) That leaf, taking into consideration gardens of the same type, which contains the most enzyme makes the most highly flavoured tea.

This increase of enzyme in the leaf seems connected in some way with the amount of phosphates in the soil.

(5) That the amount of enzyme in the leaf materially increases during withering, a fact which throws an entirely new light on the nature of the process, and makes it probable that it performs much more important functions in the manufacture than those with which it has been hitherto credited.

Other enzymes occur in the tea leaf, but I have no evidence at present that their part in the manufacture of tea is of great importance. Starch occurs in very minute proportion, and as would be expected, a small quantity of diastase with it. This starch persists throughout the withering operation but entirely disappears during the fermentation. The diastase can however be detected right through until the tea is fixed, but only in very small amount. The tests I made as to the existence of a proteolytic enzyme leave the matter in some doubt, but I certainly could get no reaction by Fermi and Buscaglioni's method with gelatine.

The Catalase of Oscar Loew * was, of course, present in rather large quantity, but I can attribute no important function in the manufacture of tea to its presence. Considering the tendency existing to form Hydrogen Peroxide in organic liquids exposed to sunlight, it seems natural to consider that it is here present to prevent the formation of this substance, which could only be a source of injury during the growth of the tea to the plant. Its presence almost exclusively immediately under the cuticle cells would materially support this hypothesis.

In conclusion, I have to thank two or three gentlemen whose assistance has been of material advantage to me in this work: These are Mr. Hooper of the Indian Museum, Calcutta, for making several analyses of materials for me, and to Mr. C. R. Newton of Kurseong, whose help in the microscopic part of the work was extremely valuable.

---

* See Report U.S.A. Dept. Agri., No. 65, 1900.